

POLLINATION ECOLOGY  
AND MAINTENANCE OF  
SPECIES INTEGRITY IN CO-  
OCCURRING *DISA RACEMOSA*  
L.f. AND *DISA VENOSA* SW.  
(ORCHIDACEAE) IN SOUTH  
AFRICA<sup>1</sup>

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ABSTRACT

The orchid *Disa racemosa* was found to be pollinated by xylocopine and anthophorine bees (*Xylocopa* and *Amegilla* spp.) at several sites in the Cape Floral Region of South Africa. A precise floral mechanism ensures that pollinaria are always attached to the middle pair of legs on the pollinator. Flowers of *D. racemosa* are nonrewarding, but nevertheless attract bees searching for new food sources. Levels of pollination and fruiting success were low, varying from 4 to 48% among the eight study populations. *Disa racemosa* is often sympatric and co-flowering with its very similar, though much rarer, sister species *Disa venosa*. The only character that is consistently different between the taxa is the width of the sepals. Although the two species appear to have the same pollinators, natural hybrids between them have never been found. The results of experimental crosses indicated that a sterility barrier, rather than ethological or mechanical barriers, is responsible for the maintenance of species integrity in mixed populations.

Coexistence of closely related species is possible only if there are effective barriers to hybridization (Levin, 1978). The orchid family is renowned for the apparent ease with which thousands of artificial hybrids have been created by hobbyists (Dressler, 1981). Yet, closely related orchids often occur in sympatry without hybridizing, and an intriguing question is how these species manage to coexist when genetic barriers to hybridization appear to be weakly developed in the family.

It is generally thought that specialized pollinator relationships and elaborate floral mechanisms in orchids prevent, or at least minimize, export of pollen to stigmas of other sympatric species (van der Pijl & Dodson, 1966; Dressler, 1981). Several studies have reported divergent pollination systems in sympatric orchid species that seldom form natural hybrids (Stoutamire, 1974; Smith & Snow, 1976; Chase, 1986; Manning & Linder, 1992; Steiner et al., 1994; Bower, 1996). Isolation of many of these species seems to be based on ethological or mechanical barriers only, since they can be crossed

easily by artificial means. However, there is some evidence that sympatric orchid species that share pollinators may occasionally possess sterility barriers, as in the case of the sympatric *Cryptostylis* species studied by Stoutamire (1975).

In this study we focus on a pair of closely related species—*Disa racemosa* L.f. and *Disa venosa* Sw.—which often occur sympatrically in the Cape mountains of South Africa. Most authorities have recognized the two species as being distinct, although Schlechter (1901) reduced *D. venosa* to a variety of *D. racemosa*, a treatment that has not been adopted by any subsequent authors (Linder, 1981).

*Disa racemosa* is one of the more common orchids in the Cape floral region, growing in marshes and seepage areas. Flowering in *D. racemosa* is strongly stimulated by fire. Hence, populations flower only at intervals of 5–30 years, which corresponds to the frequency of fires in the Cape fynbos vegetation. Flowering occurs during November and December. The inflorescence of *D. racemosa* bears from 1 to 15 pink-magenta flowers, which are

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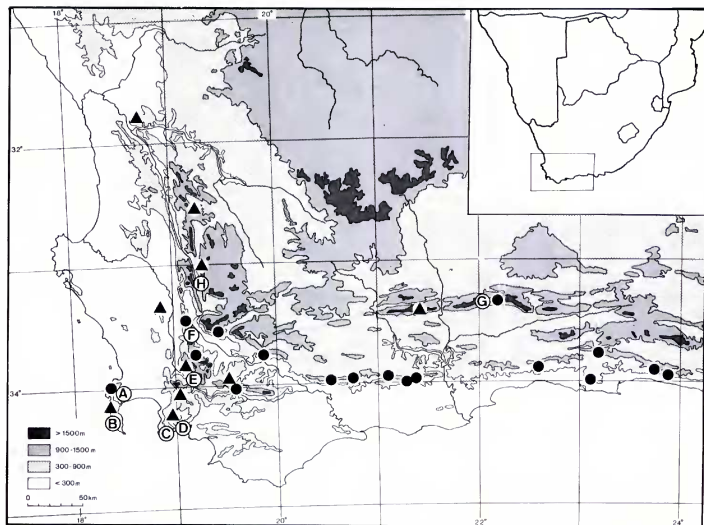


Figure 1. The distribution of *Disa racemosa* (circles) and *D. venosa* (triangles) in the Cape floristic region. The localities of the study sites are indicated by capital letters in open circles. A = Silvermine Nature Reserve, B = Cape Point Nature Reserve, C = Betty's Bay, D = Ysterklip, E = Franschoek, F = Bains Kloof, G = Swartberg Pass, H = Gydlo Pass. Populations of *D. racemosa* occur at all of the study sites, and *D. venosa* is sympatric with *D. racemosa* at sites F and G.

about 40–60 mm in diameter. The flowers do not produce nectar or any other floral reward. Most *Disa* species have a spur formed from the dorsal sepal. However, the spur in *D. racemosa* (and also *D. venosa*) is virtually obsolete, consisting of a mere shallow depression at the back of the dorsal sepal.

*Disa venosa* is remarkably similar to *D. racemosa*; the only character that consistently separates the two species is the much narrower dorsal and lateral sepals of *D. venosa* (see below). The two species have overlapping distribution ranges and are sometimes found flowering side by side in the same marshes after fire. However, *D. venosa* is much less common than *D. racemosa* and is not as often seen or collected.

Our interest in *Disa racemosa* and *D. venosa* was generated by the observation that the two species are very similar morphologically, yet appear never to hybridize, despite sharing the same habitat and flowering time. As in many other South African orchids, no previous investigation of pollination biology in the species had been undertaken.

This study had the following aims: (1) to confirm whether or not the taxa are readily diagnosable as separate species in the field; (2) to characterize the pollination biology of each species; (3) to determine the levels of pollination and fruiting success in natural populations; (4) to establish the mechanisms that allow coexistence between the species.

## MATERIALS AND METHODS

### FLORAL CHARACTERISTICS

Floral parts were measured to the nearest 0.5 mm in populations of *Disa racemosa* at Franschoek and *D. venosa* at the Bains Kloof site during 1993 (see Fig. 1 for localities of study sites). Further measurements were also made of dried specimens in local herbaria (BOL and NBG).

The flowers of *Disa racemosa* and *D. venosa* appear to have a very similar color in the field. To obtain objective measures of floral coloration in the visible spectrum (400–700 nm), we measured the reflectance of sepals of each species with an ACS

550m spectrophotometer. Bee vision is known to extend to wavelengths shorter than 400 nm, but no spectrophotometer capable of measuring ultraviolet reflectance was available. Instead, we took photographs on Tri-X film with and without a Corning 7-60 "black" filter, which only transmits ultraviolet light. The gray scale described in Kevan et al. (1973) was used to standardize exposure of prints. The photographic method has the advantage of being able to reveal floral patterns in the ultraviolet wavelengths, unlike the spectrophotometer, which takes average measurements of reflectance.

#### POLLINATOR OBSERVATIONS

Observations of pollinator visits to the flowers of *Disa racemosa* were made between 1990 and 1995 at eight sites in the Cape Floral Region (Fig. 1). Populations at these sites varied from ca. 100 plants at Betty's Bay to several thousand plants at Franschhoek. Small populations (ca. 20 plants) of *D. venosa* were found to co-occur with *D. racemosa* at the Bains Kloof and Swartberg sites (Fig. 1). At all of the study sites, the vegetation had been burnt during the previous season, thus triggering a flowering display of the orchids.

Pollinators (defined as insects that remove and deposit pollinaria of the orchids) were captured either directly on the orchids or else while foraging on nearby food plants. A herbarium voucher from the Bains Kloof population (Steiner 2274) is deposited in NBG, while the other populations are represented by existing collections in NBG and BOL. Insect vouchers are deposited in the South African Museum, Cape Town.

#### POLLINATION SUCCESS

Pollination success was measured in most of the populations of *Disa racemosa* by determining the frequency of pollinarium removal and pollen deposition on the stigmas of randomly selected samples of flowers between 1990 and 1995. We also recorded fruiting success where possible.

#### CROSSING EXPERIMENTS

Since our initial observations indicated that *Disa racemosa* and *D. venosa* may have the same pollinators, we made reciprocal crosses between the two species to determine if they are capable of hybridization. Crosses were performed between *D. racemosa* at the Franschhoek site and *D. venosa* at the Bains Kloof site. Although the two species occur sympatrically at the Bains Kloof site, no plants of *D. racemosa* flowered there in 1994, making it necessary to use plants from another site.

At each site, we covered inflorescences with pollinator-exclusion bags while flowers were still in bud. Following anthesis, flowers were randomly assigned to one of the following treatments: (1) unmanipulated to test for autogamy, (2) hand-pollinated with pollinaria from conspecific plants, and (3) hand-pollinated with pollinaria from the sister species. To make the latter crosses, we transported freshly cut inflorescences between the sites, a distance of ca. 30 km, and withdrew pollinaria from the anthers immediately before the hand-pollinations. This method ensured that all pollinaria used in the experiment were in optimal condition. All the crosses were made on 29 November 1994, and the fruits were harvested on 31 December 1994 before dehiscence had taken place.

To test seed viability, we used standard tissue culture procedures that have been found to work well for germinating seeds of *Disa* species. Before opening each fruit, we sterilized the outside with 10% sodium hypochlorite to minimize the chances of fungal and bacterial infection. The fruits were then opened in a laminar flow cabinet and the seeds from each fruit placed in separate 50-ml tissue culture flasks containing a sterile agar-based nutrient medium. The medium consisted of ¼-strength MS solution (George & Sherrington, 1984) fortified with 20g banana per litre and 2g peptone per litre. The flasks were placed in a dark cabinet for three months, followed by a 12 hr light/12 hr dark cycle at 20–25°C. Germination and development of protocorms were noted in some flasks 3–5 months after the commencement of the experiment. Seeds that had not germinated after 12 months were considered to be unviable.

#### RESULTS

##### MORPHOLOGY

*Disa racemosa* and *D. venosa* can be distinguished by the narrower sepals of *D. venosa*. Analysis of existing herbarium specimens of the two taxa showed a bimodal distribution of the dorsal sepal width/length ratio (Fig. 2). More detailed measurements of floral dimensions in populations of *D. racemosa* and *D. venosa* confirmed that the absolute width of the sepals differs markedly between the species (Table 1). The flowers of *D. venosa* at Bains Kloof were slightly smaller than those of *D. racemosa* at Franschhoek, resulting in statistically significant differences in the means of several characters (Table 1). However, sepal width was the only character that showed no overlap in the range of measurements from individuals of the two species (Table 1).

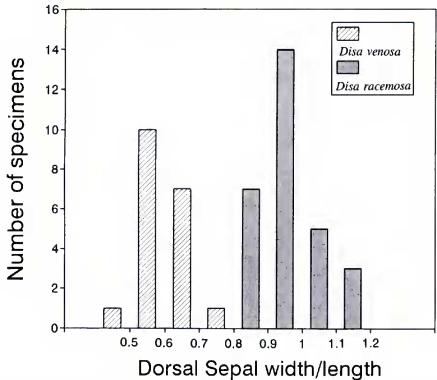


Figure 2. Frequency distribution of dorsal sepal width/length in a sample of herbarium specimens of *Disa racemosa* and *D. venosa*. Dorsal sepal width/length is the main diagnostic character separating the two species.

SPECTRAL REFLECTANCE

The reflectance spectra of flowers of *Disa racemosa* and *D. venosa* are remarkably similar, supporting the impressions gained in the field. The magenta-pink color results from strong reflectance of blue and red wavelengths (Fig. 3). The magenta-pink coloration is due to anthocyanin pigments in *D. racemosa* and *D. venosa* (Vogelpoel et al., 1985) and is a common color among bee-pollinated flowers in the Cape and elsewhere (e.g., Thien & Marks, 1972; Nilsson, 1983).

Photographs of the flowers with ultraviolet light showed that the petals, labellum, and rostellum in

both species are UV-absorptive, forming a contrast with the relatively UV-reflective sepals (Fig. 4C, D).

POLLINATOR OBSERVATIONS

Our observations indicated that *Disa racemosa* and *D. venosa* are both pollinated by medium-sized anthophorid bees and large xylocopine bees. Carpenter bees (*Xylocopa ruftarsus* Lepeletier and *X. caffra* L.) were observed to visit *D. racemosa* at five of the sites (Silvermine, Theewaterskloof, Betty's Bay, Bains Kloof, and Swartberg Pass). Smaller anthophorid bees (*Amegilla niveata* and *Amegilla spilotoma*) visited *D. racemosa* at the Swartberg and

Table 1. Measurements of floral characters in *Disa racemosa* (Franschhoek population) and *D. venosa* (Bains Kloof). All units are millimeters. NS. = not significant.

Character	<i>Disa racemosa</i> (n = 10 plants) x ± S.D. (range)	<i>Disa venosa</i> (n = 8 plants) x ± S.D. (range)	t	P
Dorsal sepal length	20.4 ± 1.9 (17–24)	19.4 ± 1.4 (18–22)	1.35	NS.
Dorsal sepal width	19.7 ± 1.8 (15–21)	9.3 ± 0.7 (9–10)	17.39	***
Lateral sepal length	22.1 ± 2.2 (18–26)	20.0 ± 1.0 (19–21)	2.70	*
Lateral sepal width	14.2 ± 2.3 (10–18)	9.0 ± 0.0 (8–10)	6.65	***
Lip length	11.8 ± 1.4 (10–14)	9.5 ± 0.7 (8–10)	4.54	***
Petal length	12.1 ± 1.5 (10–15)	10.3 ± 0.7 (9–11)	3.69	**
Distance between viscidia	4.4 ± 0.4 (4.5–5)	4.3 ± 0.4 (4–5)	0.73	NS.
Distance from rostellum to top of stigma	8.8 ± 0.8 (7–10)	8.1 ± 0.6 (7–9)	2.24	NS.
Length of pollinaria	10.0 ± 0.0	10.0 ± 0.0	0.00	NS.

\* P < 0.05, \*\* P < 0.01, \*\*\* P < 0.001.

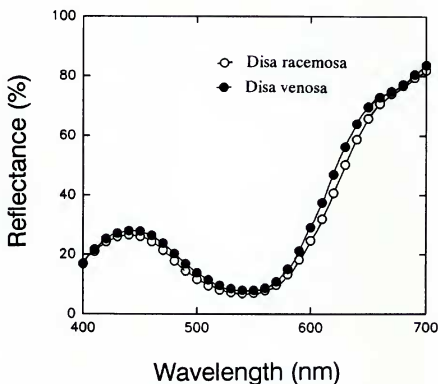


Figure 3. Reflectance spectra for *Disa racemosa* and *D. venosa*.

Bains Kloof sites. All of these bees except *X. caffra* (which may be too large to act as a pollinator) carried pollinaria attached to their middle legs. The rarity of *D. venosa* made it difficult to observe pollination in this species. A single carpenter bee (probably *X. rufitarsus*) was seen to visit flowers of *D. venosa* and *D. racemosa* in succession at the Swartberg Pass site. The behavior of the bee was identical on flowers of *D. venosa* and *D. racemosa*.

Since the orchids in this study have no floral rewards, the bees obviously need to rely on other plants in the community for pollen and nectar requirements. The observation that most floral visitors to *D. racemosa* are female bees (Table 2) suggests that the orchid primarily exploits pollen-seeking insects. Pollen-rewarding flowers that occurred at the same sites as *D. racemosa* included pink-flowered *Chironia jasminoides* L. (Gentianaceae) and *Drosera regia* Stephens (Droseraceae). Flowers of *C. jasminoides* are buzz-pollinated by female *Xylocopa* bees (S. Johnson; unpublished). At Swartberg, however, the primary food source for the smaller *Ameigilla* bees was *Moraea ramosissima* (L.f.) Druce (Iridaceae), a nectar-producing species with yellow flowers.

#### FUNCTIONAL MORPHOLOGY

Pollinaria of *Disa racemosa* were consistently attached to the middle pair of legs on the bees, implying a precise interaction between the morphology of the bees and the flower. Unlike other *Disa* species, where the dorsal sepal forms a galeate

chamber, and a spur in some species, the dorsal sepal of *D. racemosa* is almost flattened and apparently serves no function other than visual attraction. The flowers of *D. racemosa* and *D. venosa* differ from those of most other *Disa* species in having a floral chamber formed by the petals, rather than the dorsal sepal.

When alighting on a flower of *Disa racemosa*, bees grasp the petals and insert their heads forcibly into the floral chamber (Fig. 5A, B). The bees are presumably attracted to this part of the flower by the contrast between the strongly UV-absorptive petals and the relatively UV-reflective sepals (Fig. 4). In addition, the inner surface of the petals has an alternating pattern of dark and light stripes that may function as "nectar guides." While settled on the flower, the bees clasp the petals with their front legs, while the middle legs rest across the rostellum and the back legs are placed on the lateral sepals. Pollinaria become attached to the basal segment of the middle legs by means of a large sticky viscidium. It was interesting that pollinaria were always attached to the middle legs, regardless of the great variation in bee size. There appears to be space for just one pollinarium on the first segment of each middle leg, as none of the captured bees carried more than two pollinaria (one per leg), despite being observed to visit several flowers in a sequence. After withdrawal from the anther, the pollinaria are positioned so that the tip is correctly angled to strike the stigma, which is tucked underneath the projecting rostellum. The sectile pollinaria remain

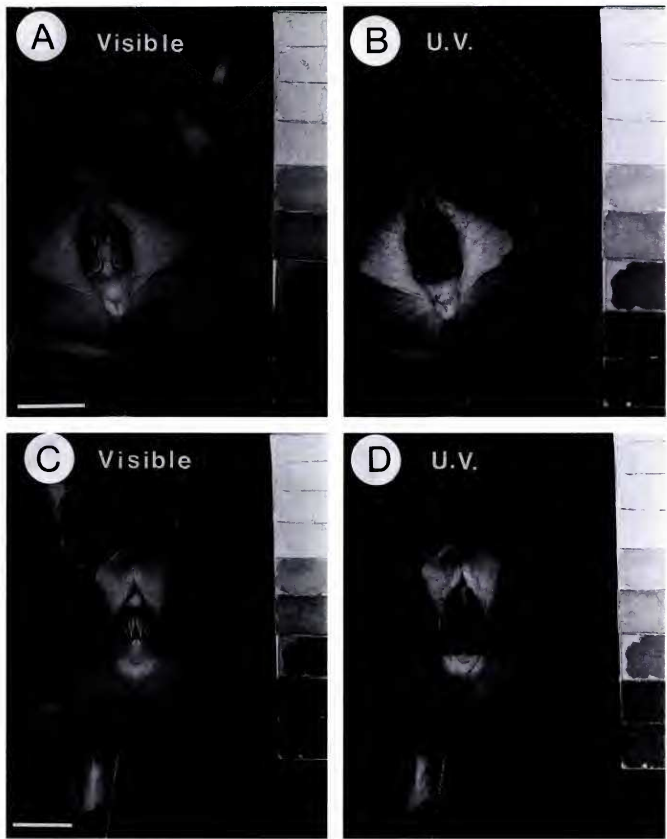


Figure 4. Comparison of reflectance of flowers of *Disa racemosa* and *D. venosa* in both visible and ultraviolet light. The gray scale is used to ensure a comparable range of contrast in each photograph. A, B *Disa racemosa*, C, D *Disa venosa*. Scale bar = 10 mm.

attached to the bees and gradually become worn as massulae are torn away from the tip after each contact with a stigma (Fig. 5D, E).

POLLINATION SUCCESS

Bees were relatively uncommon at most of the sites, except Swartberg Pass where seven bees were

caught in two days. The paucity of pollinator visits was reflected in the low levels of pollination and fruiting success, which varied between 4% and 48% (Table 3). The median level of pollination success in seven populations was only 13.6%, while the median level of fruit set in a smaller sample of four populations was 30.3% (Table 3). Since orchid



Table 2. Bee species that visited flowers of *Disa racemosa* at the study sites.

Study site	Observation time (hrs.)	Floral visitors to <i>D. racemosa</i>	Sex and pollinarium load	Food plants for the bees (P = pollen source, N = nectar source)
Swarberg	ca. 15	<i>Xylocopa ruftarsus</i> Lepeletier	♂ (1)	—
		<i>Amegilla niveata</i> (Friese)	♀ (2), ♀ (2)	<i>Moraea ramosissima</i> (N)
		<i>Amegilla spilostoma</i> (Cameron)	♀ (0), ♀ (2), ♀ (1), ♀ (2)	<i>M. ramosissima</i> (N)
			♀ (2), ♂ (0)	<i>Drosera regia</i> (P)
Bains Kloof	ca. 15	<i>X. ruftarsus</i>		<i>Chironia jasminoides</i> (P)
		<i>Xylocopa caffra</i> L.	♀ (0)	—
Silvermine	ca. 10	<i>X. ruftarsus</i>	♀ (2), ♀ (0)	—
Franschhoek	ca. 10	<i>X. ruftarsus</i>	♀ (0)	—
Betty's Bay	ca. 5	<i>X. caffra</i>	♀ (0)	<i>M. ramosissima</i> (N)
Gydo Pass	ca. 5	<i>A. spilostoma</i>	♀ (2)	—

flowers are long-lived (ca. 7–14 days in *D. racemosa*), “snapshot” measures of pollination success may lead to an underestimate of the final levels of fruit set.

#### HYBRIDIZATION EXPERIMENTS

Flowers that were bagged and left unmanipulated did not form fruits, indicating that both species are incapable of autogamy. Both intra- and interspecific crosses resulted in the formation of well-developed fruits with seeds. While seeds resulting from intra-specific crosses germinated and formed vigorous seedlings after five months, seeds resulting from crosses between *D. racemosa* and *D. venosa* showed no signs of germination after twelve months (Table 4).

#### DISCUSSION

##### POLLINATION BY DECEPTION

The observations reported in this study show that *Disa racemosa* is pollinated by xylocopine and anthophorine bees that visit the flowers even though they do not contain a floral reward. The large pink floral display seems to be sufficient to attract bees that enter the general vicinity of the population. These bees are probably sampling potential new food sources and after probing a few empty flowers they usually fly off again. There is no compelling evidence that *D. racemosa* is a mimic of other rewarding species, although the flowers do bear a general resemblance to pink buzz-pollinated flowers, such as *Chironia jasminoides*, which was sympatric with the orchid at two of the sites. The pollination system of *D. racemosa* and *D. venosa* can best be characterized as generalized food-source deception (Ackerman, 1981, 1983; Boyden, 1982; Dafni, 1984; Nilsson, 1992).

The *Disa racemosa*–*D. venosa* pair have interesting similarities to many of the bumblebee-pollinated northern hemisphere orchids. The most striking similarity is the possession of large pink-magenta flowers (Thien & Marks, 1972; Nilsson, 1980, 1983; Fritz, 1990). A characteristic that appears to be shared by all deceptive orchid species is a very low level of pollination success (Nilsson, 1980; Boyden, 1982; Nilsson, 1983; Ackerman, 1986; Gill, 1989; Fritz, 1990). This may be a consequence of insects learning to avoid the unrewarding flowers (Nilsson, 1992). The low levels of fruit set in *D. racemosa* are clearly due to pollen-limitation, as supplemental hand-pollinations in two populations led to significant increases in fruit set at a whole plant level (Johnson & Bond, 1997). This was most pronounced in the Franschhoek population, where hand-pollination led to an increase in fruit set from 4% of the flowers in control plants to 63% of the flowers in hand-pollinated plants (Johnson & Bond, 1997).

##### MECHANISMS OF COEXISTENCE IN *DISA*

A plethora of artificial hybrids has been made between the species of *Disa* sect. *Disa*, to which *D. racemosa* and *D. venosa* belong (Vogelpoel, 1992 and references therein; Linder, 1990). *Disa racemosa* has been successfully crossed with several other *Disa* species, including *Disa uniflora* Berg, *D. cardinalis* Linder, *D. atricapilla* (Harv. ex Lindl.) H. Bolus and *D. bivalvata* (L.f.) Durieu & Schinz (Vogelpoel et al., 1985; Wodrich, 1995). *Disa venosa* has been successfully crossed with *D. cardinalis* and *D. tripetaloides* (L.f.) N. E. Br. (Vogelpoel, 1992).

Geographical, seasonal, and ethological barriers probably explain why genetically compatible *Disa* species seldom hybridize in nature. *Disa racemosa*

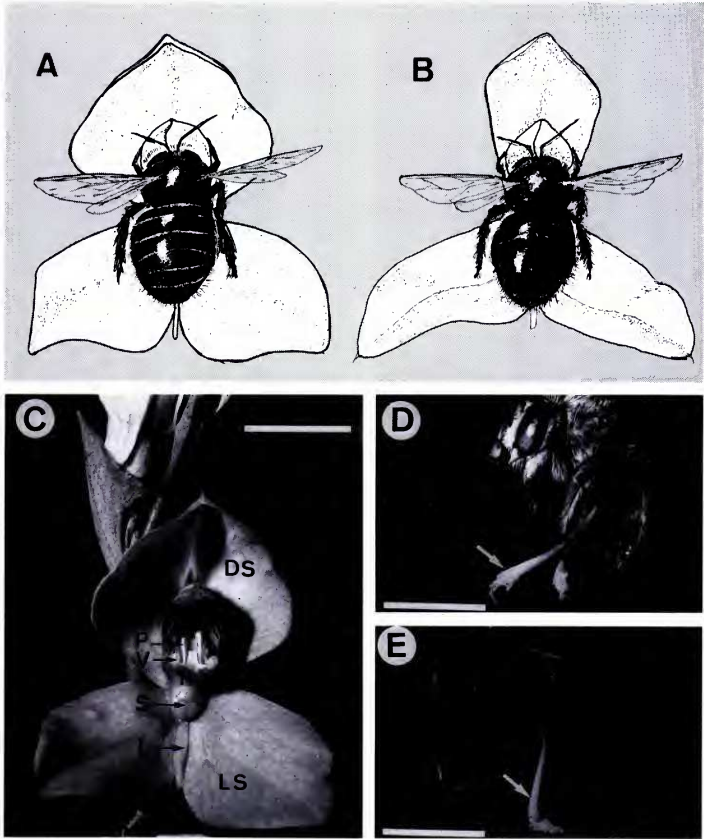


Figure 5.—A. Carpenter bee (*Xylocopa rufitarsus*) settled on a flower of *Disa racemosa*. The front legs are used to grasp the petals, the middle legs are placed over the rostellum, and the back legs are resting on the lateral sepals. The bee's head is inserted into the chamber formed by the petals. —B. Carpenter bee settled on a flower of *D. venosa*. —C. Floral morphology of *D. racemosa*. Abbreviations: DS = dorsal sepal, PT = petal, P = pollinarium, V = viscidium, R = rostellum, S = stigma, L = lip, LS = lateral sepal. Scale = 10 mm. —D. *Amegilla niveata* (Anthophorinae) with two pollinaria of *D. racemosa* attached to its middle legs. Scale = 5 mm. —E. *Xylocopa rufitarsus* (Xylocopinae) with a single attached pollinarium of *D. racemosa*. Scale = 5 mm.

and *D. uniflora*, for example, are highly interfertile, but have different habitats and pollinators; *D. uniflora* is pollinated exclusively by butterflies (Johnson & Bond, 1994), while *D. racemosa* is pollinated

by anthophorine and xylocopine bees (this study). A possible natural hybrid between *D. racemosa* and *D. atricapilla* (a wasp-pollinated species, see below) was discovered recently (Wodrich, 1995).



Table 3. Pollination and fruiting success in populations of *Disa racemosa* and *D. venosa*. Median values are given in bold type.

Study site	Date	Number of flowering individuals	Flowers with pollinaria removed % (n)	Flowers with pollen on the stigma % (n)	Flowers that set fruit % (n)
<i>Disa racemosa</i>					
Franschhoek	Dec. 1994	ca. 2000	4.9 (41)	7.3 (41)	4.1 (48)
Bains Kloof	Jan. 1991	ca. 250	—	33.9 (115)	45.3 (589)
Cape Point	Dec. 1991	ca. 100	22.2 (81)	4.9 (81)	—
Silvermine	Nov. 1992	ca. 100	33.8 (59)	10.1 (59)	38.6 (210)
Swarberg	Dec. 1992	ca. 400	63.6 (44)	47.7 (44)	—
Ysterklip	Jan. 1992	ca. 50	—	—	21.9 (160)
Betty's Bay	Dec. 1993	ca. 40	4.8 (42)	9.5 (42)	—
Gydo Pass	Jan. 1995	ca. 150	23.8 (88)	17.0 (88)	—
			<b>23.0</b>	<b>13.6</b>	<b>30.3</b>
<i>Disa venosa</i>					
Bains Kloof	Nov. 1993	ca. 30	49.1 (57)	19.2 (57)	—

Steiner et al. (1994) showed that the rarity of natural hybrids between *Disa binalvata* and its sympatric sister species *D. atricapilla* can be attributed to ethological factors. Although these sexually deceptive orchids are interfertile, they are pollinated by different wasp species, thus preventing the formation of hybrids. The formation of occasional hybrids was attributed to beetles that visit the two species indiscriminately.

By contrast, no ethological barriers appear to exist between *Disa racemosa* and *D. venosa*. These species have very similar flowers with closely matched reflectance spectra. They appear to share pollinators and, importantly, their column and pollinarium morphology is identical, thus ruling out the possibility of mechanical barriers to hybridization. In the absence of ethological or mechanical barriers between these species, the only plausible explanation for the lack of natural hybrids is a sterility barrier. This hypothesis was supported by the crossing experiments. Crosses between *D. racemosa* and *D. venosa* resulted in seeds that failed to germinate, while seeds resulting from intraspecific

crosses in the same populations germinated readily to form healthy seedlings (Table 4).

There is little other evidence for sterility barriers among closely related orchids. Stoutamire (1975) found that several sympatric *Cryptostylis* species in Australia share the same wasp pollinators without forming natural hybrids. Crossing experiments suggested that a sterility barrier may prevent hybrid formation. Genetic barriers are known to occur among less closely related orchids. Nilsson (1980), for example, showed that natural hybridization between bumblebee-pollinated *Dactylorhiza sambucina* (L.) So6 and co-flowering *Orchis* species is prevented by a sterility barrier. Dressler (1981) pointed out that it is difficult to estimate the extent of sterility barriers in the Orchidaceae, since unsuccessful attempts to hybridize species are seldom reported.

The basis for the apparent sterility barrier between *Disa racemosa* and *D. venosa* is not known. Differences in cytology can be ruled out as the two species share a diploid chromosome number of  $2n = 38$  (Pienaar et al., 1989). It is curious that while

Table 4. Results of reciprocal crosses to determine the compatibility of *Disa racemosa* and *D. venosa*. Floral measurements taken in the parent populations are given in Table 1.

Pollen recipient	Pollen donor	Number of crosses	Number of swollen fruits	Number of fruits flasked	Number of flasks with seedlings	Number of seedlings per flask
<i>D. racemosa</i>	<i>D. venosa</i>	8	8	5	0	0
<i>D. venosa</i>	<i>D. racemosa</i>	6	6	3	0	0
<i>D. racemosa</i>	<i>D. racemosa</i>	3	3	3	3	>100
<i>D. venosa</i>	<i>D. venosa</i>	7	7	3	2	>100

less closely related *Disa* species hybridize easily, these two sister species should be inter-sterile. Because of the rarity of *D. venosa*, we were not able to replicate the crossing experiments on a large scale, but it would be useful to attempt further crosses to determine if the sterility barrier between the two species is absolute or not. Since crosses between *D. racemosa* and *D. venosa* resulted in apparently normal fruits and seeds with embryos, we assume that the isolating barrier is postzygotic.

#### DIVERGENCE OF *DISA RACEMOSA* AND *D. VENOSA*

It is difficult to determine if the evolutionary divergence between *Disa racemosa* and *D. venosa* has an ecological basis. The only consistent external difference between the species is the width of the dorsal sepals. There seems to be no difference in the habitat, pollination biology, or flowering time of the two species. This situation is quite unlike that in the rest of the genus *Disa* where speciation has been clearly associated with shifts between pollinators (Johnson et al., 1998). We can only guess at the factors that promoted speciation in the *D. racemosa*–*D. venosa* pair. The width of the sepals in *D. racemosa* and *D. venosa* does not have any obvious adaptive significance for bee-pollination. Presumably this character diverged through non-adaptive processes, such as genetic drift in small isolated populations, while the overall divergence in the genomes of the two daughter species was profound enough to cause a sterility barrier. We doubt that a sterility barrier between *D. racemosa* and *D. venosa* could have arisen through natural selection, as it is difficult to imagine why a hybrid between species that share near-identical floral morphology and habitat would suffer reduced fitness in terms of pollinator attraction or seedling establishment.

Ultimately, it is difficult to determine with certainty whether a sterility barrier between congeners arose through natural selection (reinforcement), as a single mutation that preceded divergence in sympatric populations, or as a pleiotropic consequence of character divergence in allopatric populations (Grant, 1994). The present-day distribution of closely related species offers few clues about the mode of speciation. For example, although *D. racemosa* and *D. venosa* are often found sympatrically, this does not exclude the possibility that they diverged in allopatry and later expanded their ranges to become sympatric at some sites.

The findings of this paper contradict much of the current dogma about isolating mechanisms in orchids. The *Disa racemosa*–*D. venosa* pair is one of

the few known cases where sterility barriers, rather than divergent pollination systems or floral mechanisms, are responsible for species integrity of sympatric orchid species.

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